

# Bacterial Colonization of the Nasopharynx in Paediatric Patients with Acute Respiratory Viral Symptoms

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## Preface

*Streptococcus pneumoniae* is an invasive, sterile-site pathogen accounting for approximately 1500 notifications annually in Australia. This number however underestimates its clinical impact, as it does not include ear or eye infections, or pneumonias unaccompanied by septicaemia. Its interaction with humans involves asymptomatic colonization, especially of children, and this may serve as a prelude to invasive disease. This study examined colonization by pneumococci and other potentially clinically significant bacteria in children presenting with acute respiratory infections. It was found that children are very commonly colonized with bacteria which are capable of clinical disease. Similarly, they frequently carry "paediatric strains" of pneumococci which can exhibit antibiotic resistance and are capable of invasive disease. Children are therefore the likely reservoir for pneumococci, and an important factor in the epidemiology of the pneumococcus. This organism is now actively vaccinated against in Australia, and this is likely to both inhibit colonization by vaccine strains, and possibly select for serotype replacement strains. This study represents a "snapshot" of capsular strains colonizing children prior to the introduction of universal pneumococcal vaccination in Australia, what antibiotic resistance they possessed, and whether their acquisition is influenced by respiratory viral infection.



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Thanks also to Professor Lyn Gilbert who kindly allowed me to relocate to Westmead Hospital midway through this project, and continue my studies here. The same generosity of space and use of consumables was afforded to me here as was at CHW. Professor Gilbert's interest and expertise in epidemiology and molecular typing allowed for a new focus for this project in evaluating new methods for the typing and detection of pneumococci.

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## Abstract

Pneumococcal disease involves infections at both sterile and non-sterile sites, and accounts for considerable morbidity and mortality in Australia. Its public health significance has prompted the progressive introduction of vaccination against the most clinically significant capsular serotypes. Initially, vaccination involved those at greatest risk, such as indigenous Australians and those with chronic diseases and other underlying risk factors. More recently, universal vaccination was introduced for children, using immunogenic protein-conjugated capsular polysaccharide antigens of the most common invasive paediatric pneumococcal strains.

Vaccination is protective against invasive disease, but also introduces selection pressures against those vaccine strains. This will likely alter colonization dynamics, with perhaps serotype replacement or the emergence of vaccine escape mutants. Evidence to date suggests that vaccine serotypes have been partially displaced, and that some non-vaccine strains, such as 19A, are increasingly associated with invasive disease.

This study sought to establish the state of colonization by pneumococci, and other potential bacterial pathogens in children prior to the introduction of the universal 7-valent conjugated capsular polysaccharide vaccine in 2005. Children represent the reservoir for pneumococci in the community, and are known to harbour antibiotic-resistant strains. This study helps establish a colonization baseline, which may assist in detecting changes in colonization attributable to universal vaccination. The study also sought correlates between bacterial nasopharyngeal colonization, and viral respiratory infection. It has long been thought that viral respiratory infections predispose for invasive pneumococcal disease, possibly because of

co-acquisition of both virus and the bacterium in respiratory droplets, with the viral infection then producing mucosal inflammation and injury conducive to bacterial invasion.

During 2004 and 2005, 495 nasopharyngeal aspirate specimens (NPA) for children up to 5 years of age presenting with acute respiratory infections were tested. These represented those eligible for study inclusion, and did not include those excluded on the basis of age, extended time as an inpatient, or repeat collections. NPA sampling was conducted over a total of 18 months, but specimens were most frequently collected during winter and early spring in 2004 and 2005. For analysis, the participants were broken into age groups to allow comparison with previous studies. Participants comprised 130 children aged less than 6 months, 256 aged 6 months to less than 2 years, and 109 aged 2 to 5 years.

Bacterial colonization was common, with 79% of specimens growing either *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae* or *Moraxella catarrhalis*. Co-colonization was common. *H. influenzae* isolates were non-typeable. *S. pneumoniae* was present in 33% of specimens combined for age and gender, with *H. influenzae* similar (34%), followed by *S. aureus* (25%) and *M. catarrhalis* (37%). Three percent of children were colonized with methicillin-resistant *S. aureus*. *S. pneumoniae* isolates were typed for their capsular polysaccharide antigens by both established serological techniques using antisera from the Statens Serum Institut, Denmark, and by a multiplex PCR reverse line blot assay (mPCR/RLB). These 2 techniques showed very good agreement, and showed that 63% of pneumococcal isolates for all ages were vaccine strains covered by the 7-valent protein-conjugated capsular polysaccharide vaccine (4, 6B, 9V, 14, 18C, 19F and 23F). The most commonly isolated vaccine strains were 19F (49% of total), 23F (14%), 6B and 14 (both 13% of total). The most common non-vaccine strains were 6A (17% of total) and 19A (9%). Generally a single pneumococcal serotype alone was recovered – only 1 specimen yielded 2 capsular serotypes.

Antibiotic susceptibility was determined for *S. pneumoniae* isolates by E-test and disc diffusion methods, and interpreted to Clinical and Laboratory Standards Institute (CLSI) guidelines. Penicillin non-susceptibility was detected in 40% of pneumococci isolates for all ages combined. Penicillin non-susceptibility amongst the more commonly isolated 7-valent vaccine serotypes ranged from 90% of 9V isolates, to 15% of 6B isolates. Of the frequently isolated non-vaccine serotypes, penicillin non-susceptibility was detected in 67% of 19A isolates, and 18% of 6A isolates. Ceftriaxone non-susceptibility was present in 18% of all *S. pneumoniae* isolates, for all ages combined. Erythromycin non-susceptibility was found in 33% of total isolates, and clindamycin non-susceptibility in 20%.

NPA specimens were tested by immunofluorescence for the presence of respiratory syncytial virus (RSV), influenza A and B, parainfluenza viruses 1, 2 and 3, and adenovirus. Specimens were also cultured to detect less common viruses not covered by immunofluorescence, but human metapneumovirus and coronaviruses were not detectable in the study. A single virus was detected in 54% of all specimens – no specimen yielded more than 1 virus. RSV was most frequently found (148 in total, representing 57% of all viruses). Rhinoviruses were the next most frequent (16.1%) followed by adenoviruses (11.5%), influenza A (7.3%), parainfluenza 3 (4.2%), parainfluenza 1 (2.3%), echovirus (1.1%) and enteroviruses (0.8%). Only one specimen tested positive for influenza B.

Statistical tests for association between respiratory viruses and the presence of colonizing bacteria gave mixed results. Chi-squared testing indicated an association between the presence of *S. pneumoniae* for all ages combined, and RSV detection ( $p = 0.03$ ). This however only held for children in the age range of 6 months to less than 2 years ( $p = 0.038$ ), and not for those aged less than 6 months, or those 2 years to 5 years. *H. influenzae* was significantly associated with the detection of any virus in the NPA. No other significant

association between bacteria and viruses were detected by the Chi-squared test. Multiple logistic regression analysis however did not indicate any contribution by RSV to the presence of nasopharyngeal *S. pneumoniae*. In contrast, a significant contribution by *H. influenzae* and *M. catarrhalis* was found (*p* values 0.02 and 0.014 respectively). No other associations approached significance.

This study confirms previous findings that children have high rates of nasopharyngeal colonization with *S. pneumoniae* and other potentially pathogenic bacteria. The capsular strains of pneumococci isolated are largely identical to those reported in childhood colonization studies from Europe and the USA. Other strains are less frequent colonizers. Coverage of the strains isolated by the current paediatric vaccine only extends to approximately 63%. Of potential concern is the presence of serotype 19A, a strain which has been more frequently isolated in invasive disease overseas in the post-vaccine era. Antibiotic resistance was quite common in the pneumococci isolated. Viral respiratory infection was commonly superimposed on pneumococcal colonization, although the statistics were discordant on the strength of association. The study establishes a snapshot of the colonization, and antimicrobial resistance present in a population at the commencement of universal vaccination. Vaccination perturbs pneumococcal colonization biology, so the potential now exists for strain displacement and vaccine-escape variants to become more frequent colonizers, and possibly more commonly invasive and clinically significant. The study details a reverse line blot (RLB) method to better track colonizers, and provides the colonization baseline against which changes may be compared.



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## Abbreviations List

ABS	Australian Bureau of Statistics
AOM	Acute otitis media
CHW	Children's Hospital at Westmead
CLSI	Clinical and Laboratory Standards Institute
CSF	Cerebrospinal fluid
DFA	Direct immunofluorescent antibody testing
dNTP	Deoxynucleotide triphosphate
ECL	Enhanced chemiluminescence
<i>gyrA</i>	DNA gyrase gene
HiB	<i>Haemophilus influenzae</i> serogroup B
IgA	Immunoglobulin A
IPD	Invasive pneumococcal disease
<i>lytA</i>	Autolysin gene
MIC	Minimum inhibitory concentration
MLEE	Multilocus enzyme electrophoresis
MLST	Multi-locus sequence typing
mPCR	Multiplexed polymerase chain reaction
mPCR/RLB	Multiplexed polymerase chain reaction / reverse line blot assay
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MSA	Mannitol salt agar
NPA	Nasopharyngeal aspirate
N	Normal molarity

OM	Otitis media
<i>parC</i>	DNA topoisomerase IV gene
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
PFGE	Pulse field gel electrophoresis
<i>Ply</i>	Pneumolysin gene
RAP-PCR	Ribonucleic acid arbitrarily primed polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RLB	Reverse line blot assay
RNA	Ribonucleic acid
RSV	Respiratory Syncytial Virus
SMG	Skim milk glucose glycerol broth
UK	United Kingdom
USA	United States of America
<i>wzy</i>	Capsular polysaccharide polymerase gene
7vPCV	7-valent pneumococcal conjugate vaccine
23vPPV	23-valent pneumococcal polysaccharide vaccine